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Abstract

Human thyroid cancer cells in the pleural effusion were serially cultivated in vitro. Three kinds of cell lines were established from the same primary culture and were designated as PS, TS and TR lines, respectively. These three have been cultured for 574 days up to May 1, 1968. The cells of PS and TR lines were epithelial-like, whereas those of TS line revealed fibroblastic character. The chromosome numbers of PS and TR lines exhibited the modes near the hypertetraploid region, while TS line showed the mode of hypo-triploid number. Eosinophilic particles which were stained metachromatically by toluidine blue were present in the cytoplasm of these cells. The histochemical findings of the cells of each line were identical with those of thyroid cancer cells in vivo. The cells aggregated by the gyratory culture showed epithelial characters under microscopic observation of the sectioned specimens. The tumors produced in conditoned hamsters demonstrated undifferentiated cancer, which resembled the metastatic thyroid cancer of the patient. Neither collagen nor argentaffine fibers were detected with Van Gieson staining or silver impregenation.

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TISSUE CULTURE OF HUMAN THYROID CANCER

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Various kinds of human tumor cell lines have been reported, since GEY and his co-workers established HeLa cell. Cultures of tumor cells derived from body fluids were also reported by several investigators(1—6). Concerning the human thyroid cancer cell line, JONES *et al.* reported the established line derived from metastatic tissue of anaplastic thyroid cancer (7). This is the only report encountered in search for the literature on the long-term culture of human thyroid cancer. Varying types of human tumor cell lines will be necessary for *in vitro* studies of cancer, especially for the screening of anticancer drugs, immunological or cytogenetical approaches to cancer.

When tumor tissues are cultured, there still arises the question whether those growing cells are originated truly from tumor cells or from normal cells constituting tumor tissues. Various efforts have been made to clarify this problem. Nutritional requirements (8, 9), metabolic characteristics (10), and histochemical studies (11) have failed to show differences between normal and neoplastic cells in cultures. The serological studies revealed species specificity of serially cultured cells, but immunological specificity of malignant character was not detected (12). Electrophoretic mobility (13) and heterologous transplantability (14) differed significantly between normal and neoplastic cells, but these differences were not so conclusive, yielding some exceptions. When tumor cells contained in body fluid are cultured, on the other hand, the identification of cultured cancer cells is relatively easy, in the case that the growing cells demonstrate quite different characters from the ones of the cells cultured from non-cancerous body fluid. Establishment of the cell lines from pleural fluid of a patient of thyroid cancer was attempted from this point of view. Morphological observations, histochemical approaches, gyratory culture, chromosome analysis, and hetero-transplantations were carried out for the determination of the malignancy of the cultured cells.

MATERIALS AND METHODS

Cells ana culture methods: Pleural effusion was derived from a 58-year old patient of thyroid cancer, who died 18 days after the initiation of the culture. Undifferentiated carcinoma of thyroid gland and numerous metastases to the lymph nodes of pleural cavity were detected at the autopsy. The pleural effusion was examined under a microscope by Giemsa staining immediately after the collection. Many lymphocytes and large tumor cells were observed. The tumor cells contained a single, large nucleus or sometimes many nuclei of irregular size. Polymorphonuclear leukocytes and a small number of macrophages were also present.

Cells were collected by the centrifugation at 1,000 rpm for five minutes and about 12.8×10^4 cells per ml were suspended in the culture medium. For the primary culture, 2ml of the cell suspension was inoculated into ordinary test tubes and incubated at 37°C. The cells were divided into three lines after the first subculture. Two lines which were subcultured by pipetting (PS line) and with trypsin (TS line), respectively, were subjected to stationary culture. Another line was subcultured with trypsin and maintained in rotatory culture (TR line). The replacement of culture medium was done twice a week. For the estimation of cell proliferation, the simplified replicate tissue culture method was employed (15).

Culture media: Two kinds of media were used for the primary culture, the one was Earle's solulion supplemented with 20 per cent bovine serum, 0.4 per cent lactalbumin hydrolysate, and 0.08 per cent yeast extract (YLE). The other consisted of 20 per cent bovine serum, 0.4 per cent lactalbumin hydrolysate, and buffered saline (LE). Penicillin was added to the media at a final concentration of 100 units per ml. The latter medium was used for the establishment of the cell lines. The reason will be described later.

Preparation of specimens: Giemsa, mucicarmine, toluidine blue, Sudan III, and Van Giesons stainings, Gomori's silver impregnation, and PAS reaction were performed with the cells cultured on cover-slips as monolayer, after the cells were fixed with 100 per cent methanol, Carnoy's solution or 10 per cent neutral formol.

Histochemical stainings: Cultured cells of PS and TS lines were stained by the following techniques; acid phosphate by GOMORI's (16), glucose-6-phosphate dehydrogenase (G-6-PDH) and lactic dehydrogenase (LDH) by NACHLAS, WALKER and SELIGMAN (17, 18), succinic dehydrogenase (SDH) by NACHLAS, TSOU and SOURA (19), adenosine triphosphatase (ATPase) by WACHSTEIN and MEISELS (20), and alkaline phosphatase by TAKAMATSU's method (21).

Gyratory culture of cells: Cultured cells of PS and TS lines were collected by treating with trypsin. Cell suspensions containing 10^6 cells in 3 ml culture medium were rotated on a gyratory shaker by the standard procedure described by MOSCONA (22) and KURODA (23). After 24 hours of rotation aggregates were examined with microscope, fixed with Carnoy's fixative and prepared for histological examinations.

Chromosome analysis: Colchicin was added to the medium for 2 to 6 hours at a final concentration of 1γ per ml. Then the cells were harvested with trypsin or pipetting, mildly centrifuged and washed twice with 0.85 per cent NaCl solution. The hypotonic treatment of the cells was done with 1 per cent sodium citrate. After 10 to 15 minutes of the treatment, 2 to 5 drops of acetic methanol (1 : 3) were added without agitation and the materials were kept at cool temperature. An additional fixative was placed over the cell suspension and the mixture was agitated gently. After 5 minutes 1 to 2 drops of the fixed materials were dropped on the slides soaked in 50 per cent ethanol and air dried over weak frame. The specimens were stained with 7 per cent aceto-orcein or 5 per cent Giemsa solution.

Heterotransplantation to the cheek pouches of conditioned hamsters: Hamsters under 4 weeks of age were conditioned with 3mg of cortisone acetate 24 hours before the transplantation or with irradiation of 500r of *x*-ray just before the transplantation. Cells were harvested with trypsin, washed once with culture medium and resuspended into the medium in which the cells had been incubated. The cells were inoculated into the cheek pouches on both sides with the methods described by HANDLER and FOLEY (24). After the transplantation 2.5 mg of cortisone acetate were injected subcutaneously and the same doses were added every two days until they were sacrificed for histological examination.

RESULTS

1. *Primary culture*

The cultivation was initiated on October 7, 1966. The cells adhering to the glass wall could not be identified precisely during the first two days after the initiation of culture, because of the presence of many erythrocytes in culture. After the first replacement of medium, a small number of cells were observed adhering to the glass surface near the bottom of the test tube.

The cultured cells were composed of small round lymphocytes, oval middle-sized cells, relatively small spindle-shaped cells and large or middle-sized irregular cells. The cells grew gradually in LE medium but not in YLE medium. The cell growth in LE medium with that in YLE medium during the first 7 days in the primary culture was compared, as illustrated in Fig. 1. Since the yeast extract supplemented medium proved to be toxic to cell growth, only LE medium was used thereafter. After a week lymphocytes were lost and the growing cells varied in size and shape. However, middle-sized cubic, oval or polygonal cells and relatively small triangular to spindle shaped cells became gradually predominant, with large mono- or multinucleated giant cells still remaining. On the 33rd culture day the first subculture was performed by pipetting the half of the cells adhering to the glass surface. This was designated as PS line. The

other half of the remaining cells was submitted to subculture with trypsin on the 44th culture day and divided into two lines, TS and TR.

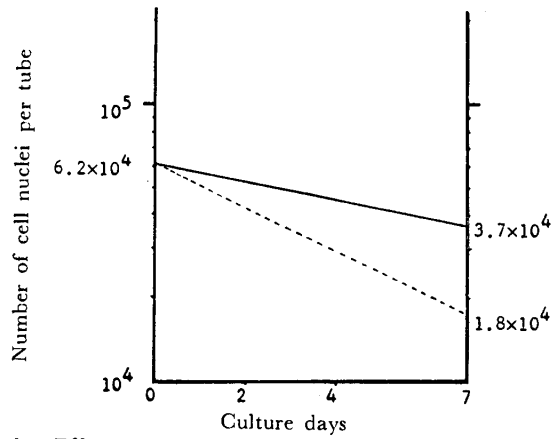

Fig. 1. Effect of the addition of yeast extract into the LE medium on the survival of thyroid cancer cells in the primary culture.
—— : LE medium ····· : YLE medium

2. *Tissue culture history and growth rate*

The culture history of three lines is summarized in Tables 1, 2, and 3. The cells of these lines are now still steadily growing. Throughout the culture history, cells were not connected firmly to one another and were easily suspended by mechanical irritation or by trypsinization.

The growth rate of each line was examined during 11th to 20th passage. The growth rate was nearly similar in three lines and the doubling time was approximately 40 hours in the exponential proliferation.

3. *Effects of bovine serum concentration and human serum on the growth*

The effect of bovine serum concentrations, i. e., at the final concentration of 60, 40, 20, and 10 per cent respectively, on the cell proliferation of TS line was investigated by the simplified replicate tissue culture method. The best growth was observed in the medium containing 60 per cent bovine serum but no significant difference was demonstrated as in Fig. 3.

Effects of human serum and bovine serum on cell growth were examined with the same TS line cells. The medium LE containing 20 per cent bovine serum showed the best result but in the one containing 10 per cent bovine and 10 per cent human sera, the cell growth was not too poor and the numbers of cells were enough for subculturing during 7-day culture (Fig. 4). When human serum was present in the medium, the cells

Table 1. History of PS line

Sub-culture	Duration (days)	Date	Total days in culture
Primary	33	Oct. 7, '66	0
1	24	Nov. 9	33
2	26	Dec. 2	57
3	12	29	83
4	21	Jan. 10, '67	95
5	46	31	116
6	16	Mar. 16	162
7	5	Apr. 3	178
8	8	8	184
9	15	16	191
10	7	May 1	206
11	10	8	213
12	4	17	223
13	7	22	227
14	13	29	234
15	9	Jun, 11	247
16	8	20	259
17	12	28	264
18	13	Jul. 10	276
19	5	23	289
20	6	28	294
21	14	Aug. 4	300
22	22	17	314
23	11	Sept. 8	336
24	6	19	347
25	12	25	353
26	8	Oct. 7	365
27	12	15	373
28	6	27	385
29	15	Nov. . 2	391
30	26	17	406
31	15	Dec. 13	432
32	11	28	447
33	9	Jan. 8, '68	458
34	8	17	467
35	21	25	475
36	7	Feb. 14	497
37	18	21	504
38	23	Mar. 10	522
39	12	Apr. 2	545
40	6	14	557
41	10	20	563
42		30	573

Table 2, History of TS line

Sub-culture	Duration (days)	Date	Total days in culture
Primary	44	Oct. 7, '66	0
1	15	Nov. 20	44
2	14	Dec. 5	59
3	22	19	73
4	31	Jan. 10, '67	95
5	5	Feb. 10	126
6	13	15	131
7	7	28	144
8	7	Mar. 7	151
9	4	14	158
10	7	18	162
11	14	25	169
12	8	Apr. 8	183
13	22	16	191
14	9	May 8	213
15	9	17	222
16	10	26	231
17	10	Jun. 5	241
18	6	15	251
19	19	21	257
20	13	Jul. 10	276
21	4	23	289
22	7	27	293
23	9	Aug. 3	300
24	6	12	309
25	5	18	315
26	16	23	320
27	6	Sept. 8	336
28	5	14	342
29	8	19	347
30	5	27	355
31	9	Oct. 2	360
32	7	11	369
33	12	18	376
34	10	30	388
35	6	Nov. 9	398
36	5	15	404
37	4	20	409
38	7	24	413
39	7	Dec. 1	420
40	8	8	428
41	6	16	434
42	9	28	443
43	5	31	448
44	6	Jan. 5, '68	454
45	8	11	462
46	6	19	488
47	7	25	475
48	6	Feb. 1	481
49	8	7	489
50	4	15	498
51	8	19	502
52	6	27	510
53	9	Mar. 4	516
54	7	13	525
55	13	22	532
56	12	Apr. 2	545
57	6	14	557
58	9	21	563
59	6	29	572
60		May 6	578

Table 3. History of TR line

Sub-culture	Duration (days)	Date	Total days in culture
Primary	44	Oct. 7, '66	0
1	15	Nov. 20	44
2	14	Dec. 5	59
3	10	19	73
4	15	29	83
5	6	Jan. 13, '67	98
6	5	19	104
7	13	24	109
8	7	Feb. 6	122
9	8	13	129
10	6	21	137
11	19	27	143
12	7	Mar. 18	162
13	19	25	169
14	7	Apr. 13	188
15	7	20	195
16	11	27	202
17	9	May 8	213
18	5	17	222
19	10	22	227
20	10	Jun. 1	237
21	24	11	247
22	22	Jul. 5	271
23	7	27	293
24	36	Aug. 3	300
25	6	Sept. 8	336
26	5	14	342
27	6	19	347
28	12	25	353
29	8	Oct. 7	363
30	8	15	373
31	5	23	381
32	5	28	386
33	15	Nov. 2	391
34	6	17	406
35	6	23	412
36	8	29	418
37	9	Dec. 7	426
38	9	16	435
39	6	25	444
40	8	31	450
41	9	Jan. 8, '68	458
42	12	17	467
43	18	29	479
44	11	Feb. 15	497
45	16	26	508
46	10	Mar. 13	524
47	10	23	534
48	12	Apr. 2	544
49	8	14	556
50	13	May 5	564
			577

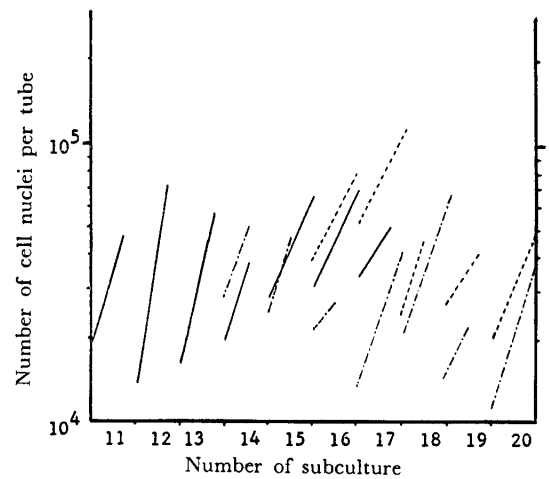

Fig. 2. The growth rate of three lines from 11th to 20th transfer generation. Each scale of the abscissa means time course of 10 days.
——: PS line ·····: TS line -·-·-·-: TR line

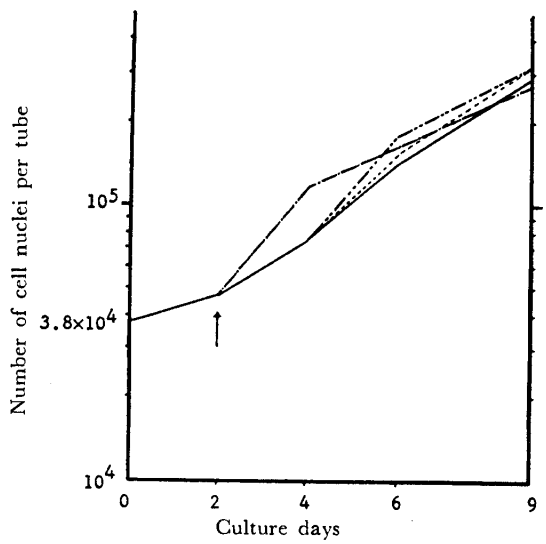

Fig. 3. Effect of the concentration of bovine serum on the survival of thyroid cancer cell (TS line) from 428 to 437 culture days. The arrow shows the start of the experiment.
-·-·-·- 60%, ------ 40%, —— 20%, -·-·-·-10%

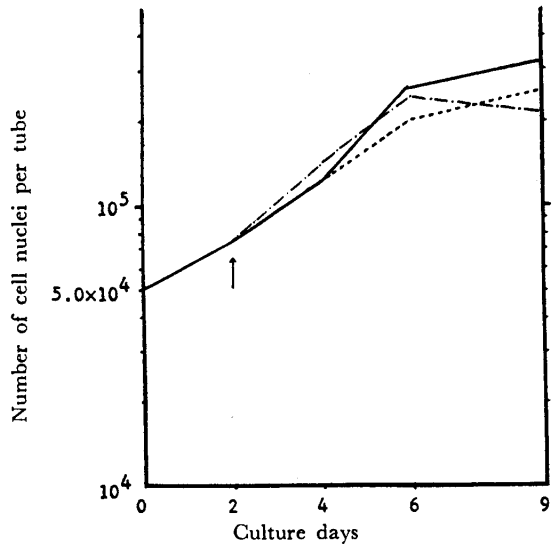

Fig. 4. The comparison in the effect between bovine and human serum on the growth rate of thyroid cancer cells (TS line) from 451 to 458 culture days. The arrow shows the start of the experiment.
——— 20% bovine serum, ------- 10% bovine serum + 10% human serum,
-·-·-·- 20% human serum

were disposed to aggregate and a few cells became pycnotic. This tendency became more striking in the medium containing 20 per cent human serum.

4. *Morphology*

In the primary culture and the first subculture the cell population consisted of three types of cells, small spindle-shaped, middle-sized oval or polygonal cells and large mono- or multinucleated cells of irregular shape.

After the 5th passage in TS line small spindle-shaped cells become predominant, whereas PS and TR lines were middle-sized oval, triangular, or polygonal epithelial-like cells. In three lines there were mono- or multinucleated giant cells.

The cells of the three lines gave negative results in mucicarmine staining and most of the cells proved negative to PAS reaction but a few cells contained PAS positive granules. Eosinophilic particles were recognized in the cytoplasm of the three lines and varied considerably in size, from the size of nucleus to that of nucleolus. These particles showed metachromatic stainability of a reddish purple color with toluidine blue. No fiber was detected by Gomori's silver impregnation or Van Gieson staining. Sudan III staining revealed no specific fatty granules. The morphological

features of three lines are shown in Photos 1, 2, and 3.

At the present writing PS line is growing in colony of pavement-like arrangement. Those cells located in the outermost border of the colony appear to be linked together turning their major axis toward circumference. These colonies gradually fuse with one another and share a glass wall as monolayer. On the other hand, TS and TR lines showed no such specific structure. Cells of three lines easily become detached from glass surface by over growth.

5. *Histochemical staining*

Acid phosphatase, G-6-PDH, SDH, LDH, and ATPase were demonstrated in some cells, especially in giant cells and multinucleated cells. G-6-PDH, SDH, and LDH were revealed in the form of fine granules in the cytoplasm put positive acid phosphatase materials were observed as large granules in the cytoplasm. ATPase was granular and chiefly on the cell membrane.

6. *Gyratory culture of the cells*

The manners of aggregation of cells of PS and TS lines were compared. When tested by rotation for 24 hours, PS line consistently formed numerous small and compact aggregates with rough surface and their diameter ranged from 0.1 to 0.3 mm (Photo 4). On the other hand, the cells of TS line showed larger, loose aggregates in undefined shapes with diameter of 0.2 to 0.6 mm (Photo 5). Histological examinations revealed that aggregated cells of PS line were polygonal in shape with eosinophilic cytoplasm and large chromatin-rich nuclei with several nucleoli (Photo 6). The cells of TS line were round or polygonal in shape and had similar characteristics to the cells of PS line in respect of eosinophilic cytoplasm and nucleoli (Photo 7). The cells of both lines had extraordinary eosinophilic particles in cytoplasm by hematoxylin-eosin staining and these particles showed reddish purple metachromasia by toluidine blue staining, which was also observed in monolayer culture and when these cells were aggregated such characteristics became more prominent than in monolayer cultured specimens.

7. *Chromosome number distributions*

Chromosome number distributions of each line were examined separately. Chromosome numbers of 50 metaphases were counted except TR line. The PS line had the modes of 100 and 103 at 17th passage (273rd culture day), and 95 and 98 at 33rd passage (461st culture day) but the peak was not so distinct. Most of the chromosome numbers of this line were distributed in hyper-tetraploid range. The TR line showed nearly the

same distributions as those of PS line. On the other hand, in TS line the peak was 66 at 19th passage (265th culture day) and 65 at 52nd passage (519th culture day) and each percentage of the peak was 20 and 26 respectively. Most chromosome numbers of this line existed in hypo-triploid range. In the three lines were observed some cells having chromosomes over 200. Endomitosis was frequent in PS and TR lines. Fig. 5 shows the chromosome number distributions of the three lines. It is to be noted that the distribution of chromosome numbers of PS and TS lines never overlaps each other, but the distribution of PS and TR lines practically coincides with each other.

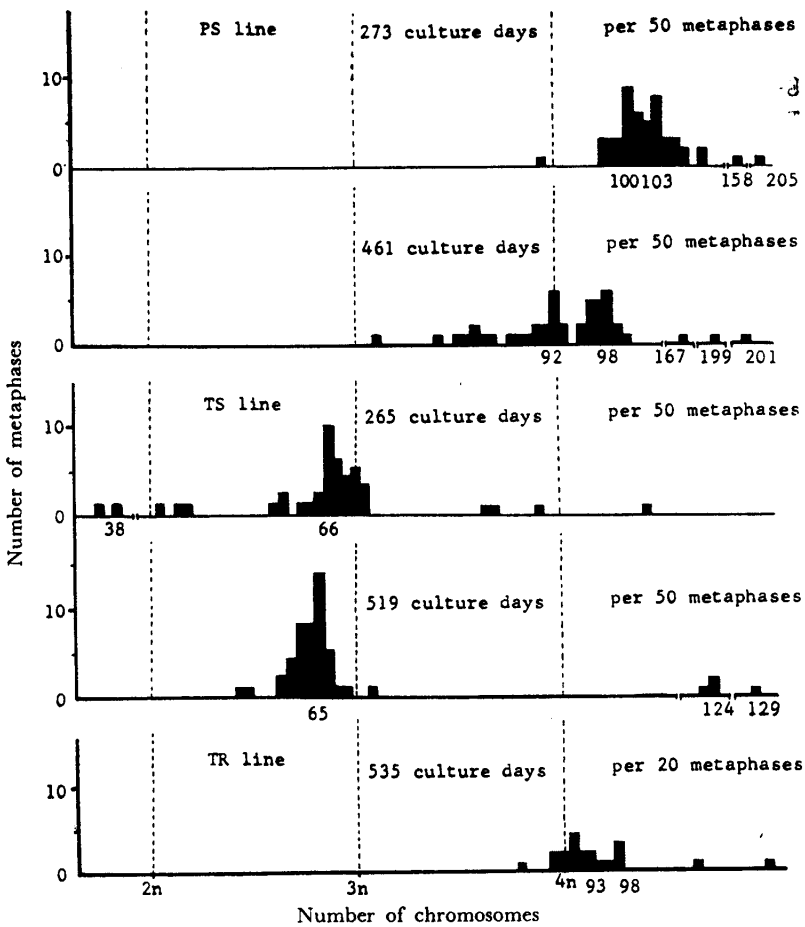

Fig. 5. Distributions of chromosome numbers in the three lines, PS, TS, and TR

8. *Heterotransplantation to the cheek pouches of conditioned hamsters*

The nodules of tumors, ranging from 0.5 to 4.5 mm in diameter, were observed two days after the inoculation at the transplanted sites. Some of them (13/30) disappeared in seven days, 7 of the 30 nodules were constant in size and 10 of the 30 grew gradually larger accompanied by vascularization, hemorrage and inflammation. Destruction of pouch epithelium and ulcer formation were observed in only one.

Histologically, cancer nestles were sometimes observed in the tumors which consisted of large polygonal cells with abundant cytoplasm having round or oval nucleus. Some nuclei showed prominently large nucleoli stained reddish by hematoxylin-eosin. The histological features of the tumors are shown in Photos 8 and 9. No fiber formation was demonstrated among tumorous cells either with Van Gieson staining or Gomori's silver impregnation. These characteristic features of heterologously transplanted tumors suggest that the cultured cells have originated from thyroid cancer (Photo 10).

DISCUSSION

The long-term cultivation of tumor tissues or tumor cells contained in body fluid presents an unavoidable problem in that the identification of the cultured cells is very difficult from their morphological or functional findings (2, 4, 25). In addition the morphology of cell lines *in vitro* differs greatly from that of original tissue cells *in vivo*. In spite of these facts the morphological observations have given one of the most important clues for the estimation of malignancy of cultured cell lines (26, 27).

When the cells in body fluids obtained from various normal or malignant diseases are cultured, there appear either epithelial-like or fibroplast-like cells (1—6, 28). However, fibroblast-like cells disapper generally in the course of successive cultures (2, 4). Concerning the three lines presented in this report, the cells of PS and TR lines were epithelial in morphology, whereas those of TS line were fibroblastic in shape. But these results do not necessarily indicate the origin of TS line to be mesenchymal cells.

The eosinophilic particles which showed metachromasia with toluidine blue seemed mucopolysaccharides produced by the cells. The meaning of the presence of these substances is not clear.

Histochemically, acid phosphatase, LDH, SDH, and G-6-PDH were present in human thyroid cancer cells *in vivo* but no alkaline phosphatase (29). Generally, these enzymes except alkaline phosphatase are said to have a tendency to be stained weaker than the enzymes of the normal epithel-

ium of thyroid gland. In the present work, all the enzymes investigated were stained in some cells but not in all. These facts seem to depend upon the culture containing various cells or upon the mitotic cycle of the cells. When these cells are cloned or cultured syncronously, these problems will be solved.

SYVERTON *et al.* reported the excellent results of cultivation of cells from normal human tissue using the medium containing yeast extract (30). On the other hand, there are some reports indicating toxic effects of yeast extract upon the primary cell growth (15). In the present experiment, it was obvious that yeast extract was not suitable for the growth at least in the case of these lines.

It is generaly accepted that the chromosome numbers of human tumor are almost heteroploidy including pseudo-diploidy (31—33). When these tumors were cultivated *in vitro*, the heteroploidy has been preserved (4, 33—35). ISING and LEVAN supposed that hypodiploid to inter diplo-triploid region was karyologically stable situation for human tumor existence (36). But some exceptional tumors which had chromosome modal numbers over tetraploid region were reported (30, 37). MOORE and KOIKE supposed that the possession of a near triploid complement of chromosomes was associated with the successful establishment of human tumor cell lines (5). As constant growth rate of these three lines was maintained from the initiation of culture, the cells transferred to the primary culture may have been of mixed population of heteroploid cells suited for the culture conditions and it is not supposed that the intense mutations which exerted influence upon the cell growth have occurred in the course of culture. HUS (32) and HANSEN-MELANDER *et al.* (38) reported the mosaic type of human tumors which showed two modes of chromosome number distributions.

Two or more modes of the distribution of chromosome numbers were reported often during the continuous culture of one cell strain. On the other hand, the relatively early appearance of the modal differences of these three lines indicated that the original tumor seemed to be mixed with the cells of heteroploid complements. As the chromosomal number distributions of TS line differed clearly from those of PS and TR lines, the TS line seems to be selected by culturel conditions other than PS and TR lines were done. LEVAN and BIESLE showed that the dispersion of cells with trypsin caused abnormal mitosis at higher frequency (39). However, it cannot be said that the differences among the cell lines lies in the effect of trypsin.

The survival of homologous transplant is considered to be the most accurate manifestation of the malignant characters of serially cultivated

cell lines from rodent. With human tumor cell lines, however, there arise some difficulties in homologous transplantation. Many investigators devised different heterologous transplantation methods to verify the malignant characteristics of biopsy specimens or cultured cell lines. Conditioning with *x*-ray irradiation (40) or cortisone treatment (14, 24, 41), special sites such as cellebrum of newborn animals (42, 43), anterior chamber of the eye (44) or chorio-allantoic membranes (45, 46, 47) permit the heterologous growth of tumor cells, though failure of such trial does not necessarily indicate the malignancy of transplanted cells. Cheek pouches of conditined hamsters are prevailingly utilized for heterologous transplantations among the above mentioned methods. Another advantage of heterologous transplantation is that histological examination of tumors growing in the transplanted sites can reveal morphological features *in vivo* and therefore, can sometimes demonstrate the origins of cells inoculated. (44). This procedure is significant especially in the cases of monolayer cultured cells, because the morphology of cells *in vitro* has been markedly changed from the cells *in vivo*.

The metastatic tumors in pleura were diagnosed as undifferentiated carcinoma of thyroid gland. The tumors consisted of pleomorphic cells having abundant eosinophilic cytoplasm, showing the specific cytoplasm of thyroid epithelial cells. The PS, TS and TR lines induced tumors in hamsters and these tumors showed the characteristics similar to the original tumor. This histological similarity of each tumor suggests that these cultured cells have originated from metastatic thyroid cancer cells. Though FOLEY *et al.* gave criteria of cell numbers inoculated for the malignancy of cultured cells (14, 35), morphological similarity may be useful for the identification of cultured cells with the original tumor cells. As the growth activity of cultured cells in heterologous animals are influenced by various factors, i. e., age, species and conditioning of animals, the numbers of the cells used in transplantation, and etc., no development of tumors does not necessarily rule out the malignancy of cultured cells.

The differences between PS and TS lines in morphology, chromosome number distributions, and aggregability in gyratory culture may lie in the fact that the original tumor itself consisted of thyroid cancer cells having different characters. These differences have been probably caused by the selection of some cancer cells or by the adaptation or the unknown factors in culture conditions, because the successful culture of cancer cells, though they have different characters *in vitro*, may be inferred from the results of the formation of similar tumors by the inoculation of the cells of both lines in conditioned hamsters.

SUMMARY

Human thyroid cancer cells in the pleural effusion were serially cultivated *in vitro*. Three kinds of cell lines were established from the same primary culture and were designated as PS, TS and TR lines, respectively. These three have been cultured for 574 days up to May 1, 1968.

The cells of PS and TR lines were epithelial-like, whereas those of TS line revealed fibroblastic character. The chromosome numbers of PS and TR lines exhibited the modes near the hypertetraploid region, while TS line showed the mode of hypo-triploid number.

Eosinophilic particles which were stained metachromatically by toluidine blue were present in the cytoplasm of these cells. The histochemical findings of the cells of each line were identical with those of thyroid cancer cells *in vivo*.

The cells aggregated by the gyratory culture showed epithelial characters under microscopic observation of the sectioned specimens.

The tumors produced in conditoned hamsters demonstrated undifferentiated cancer, which resembled the metastatic thyroid cancer of the patient. Neither collagen nor argentaffine fibers were detected with Van Gieson staining or silver impregenation.

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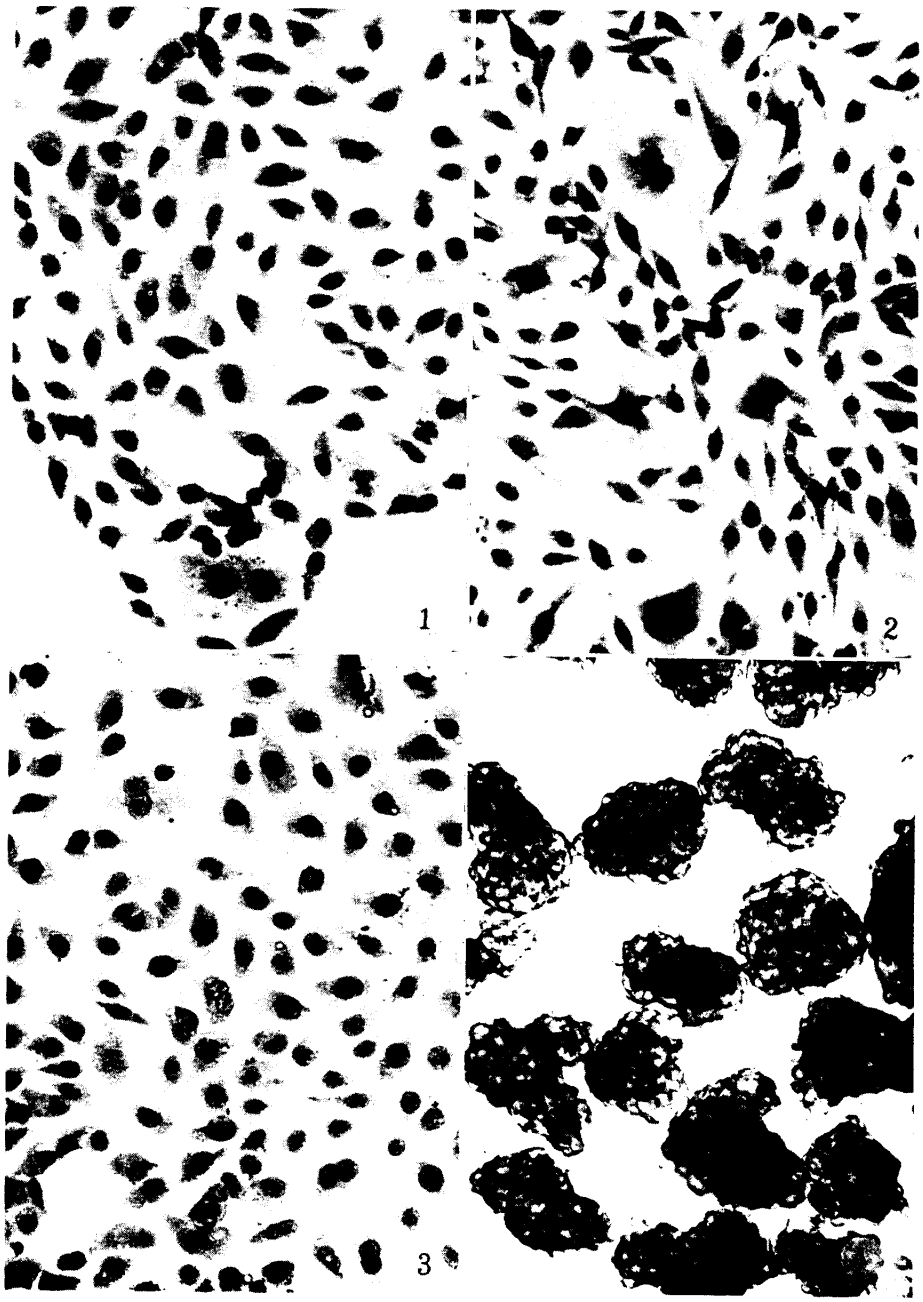
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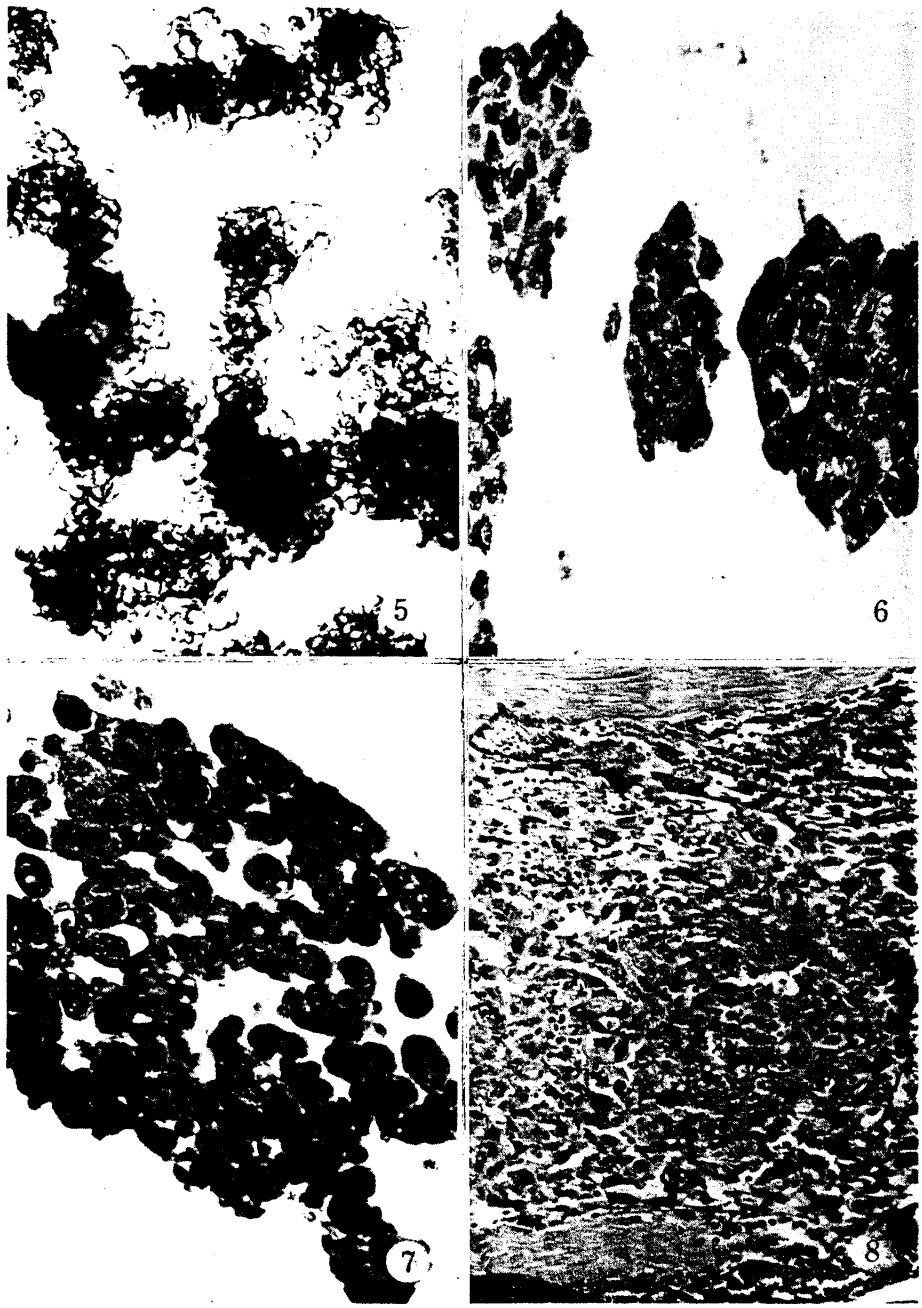
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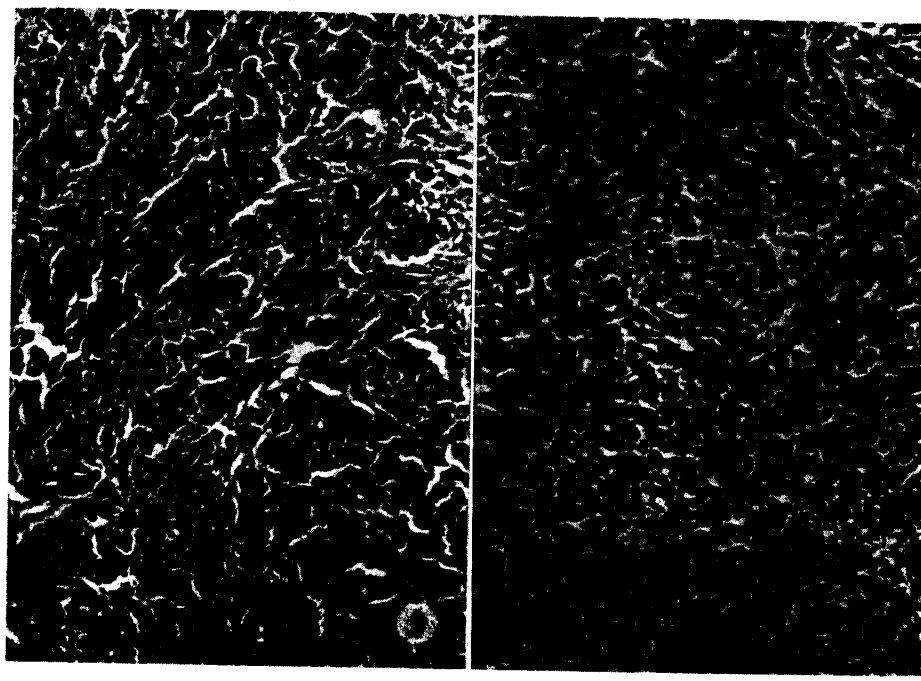

EXPLANATION FOR PHOTOMICROGRAPHS

Photo 1 The cells of the PS line at the 31st subculture after 445 days of cultivation in total. Colony formation of epithelial-like cells containing giant cells are exihibited. Giemsa staining. ×150

Photo 2 The cells of the TS line at the 37th subculture after 415 days of cultivation in total. Spindle formed cells and a few giant cells are seen. Giemsa staining. ×150

Photo 3 The cells of the TR line at the 39th subculture after 451 days of cultivation in total. Loose sheet of epithelial-like cells is shown. Giemsa staining. ×150

Photo 4 The aggregates of the PS line at the 43rd subculture after 588 days of cultivation in total. Compact aggregates with rough surface are exhibited. ×100

Photo 5 The aggregates of the TS line at the 60th subculture after 588 days of cultivation in total. Loose aggregates irregurar in shape are shown. ×100

Photo 6 Section of the cell aggregates PS line (Photo 4). Eosinophilic particles are observed in the cytoplasm of some cells. Hematoxylin-eosin staining ×300

Photo 7 Section of the cell aggregates of TS line (Photo 5). Eosinophilic particles are seen in the cytoplasma of some cells. Hematoxylin-eosin staining ×300

Photo 8 The tumor produced by the transplantation of the cells of PS line to the cheek pouch of a conditioned hamster, 6 days after post implantation. Large epithelial tumor cells are present in the central part. Hematoxylin-eosin staining ×150

Photo 9 The tumor produced by the transplantation of the cells of TS line to the cheek pouch of a conditioned hamster, 22 days after post implantation. Proliferating epithelial cells form cancer nestles in some parts. The cells show wide cytoplasm similar to Photo 10. Hematoxylin-eosin staining ×150

Photo 10 The metastatic lymphnode of the pleural cavity of the patient (undifferentiated thyroid cancer). Hematoxylin-eosin staining ×150